A STUDY OF HYBRIDS BETWEEN TWO STRAINS OF ESCHERICHIA COLI

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We have found that strain 122 (called C) of the British type culture collection recombines with strain K12S derived from strain W1294 of K12. K12S and C are sensitive to the phage λ carried by K12 (Bertani and Weigle, 1953).

Strain C differs from K12S with respect to several characters, which have not previously been studied in bacterial crosses:

(1) Cell morphology: Cells of strain C are round or oval and tend to occur in pairs more often than those of K12S. Cells of K12S are rod shaped (figures 1 and 2).

(2) Nuclear morphology: The nuclear bodies of K12S show the characteristic pattern of most bacteria and usually appear rod shaped and separated from the cell wall. The nuclear bodies of C appear smaller and globular; they seem to be more numerous and are observed at the periphery of the cell (figures 1 and 2).

(3) Host controlled modification of λ: Phage λS, produced in K12S cells, multiplies as well in K12S as in C. Multiplication in strain C modifies λS into λC, which can multiply only in very few K12S cells (Bertani and Weigle, 1953).

(4) Accepting abilities (Luria, 1953): λC and λS are accepted by and usually multiply in every C cell they infect; λC is accepted by less than 0.1 per cent of the K12S cells; λS, by every K12S cell.

(5) Strain C is sensitive to phage S13 while K12S is resistant to this phage.

We describe below the results of the crosses between K12S and C.

MATERIALS AND METHODS

A proline requiring mutant of strain C was obtained after ultraviolet (UV) treatment and selection with penicillin (Lieb, 1951). No other markers were selected in strain C. Strain K12S carried the character V_1^r (resistance to phages T1 and T5). A histidine requiring (H^-) strain and also an isoleucine-valine requiring (IV^-) strain were selected with penicillin after ultraviolet treatment. Spontaneous mutants resistant to T6 or to 100 μg per ml of streptomycin were selected in the auxotrophic K12S stocks. A lactose nonfermenting mutant (Lac^-) was selected on Difco Endo agar after ultraviolet treatment of the H^- strain.

For crossing, the parental strains were grown in broth with aeration to a concentration of about 3×10^9 cells per ml. Aliquots of the two parental cultures were mixed, and the mixtures were washed three times by centrifuging and resuspending in phosphate buffer. Parental cells washed separately were used as controls. The cells were plated in minimal synthetic medium prepared with washed agar to which the salt mixture of Gray and Tatum (1944) and 0.5 per cent glucose were added. Prototrophic colonies (recombinants) were streaked on minimal agar and single colonies restreaked on broth agar. Prototrophs were tested as follows for the characters not selected in the cross: Phage resistance was determined by "replica plating" (Lederberg and Lederberg, 1952) of the streaks to plates coated with T1 or T6. Streptomycin resistance and lactose fermentation were scored by replica plating to streptomycin agar or Endo agar. To determine the accepting abilities of prototrophs for λC and λS, the strains were grown in broth with aeration to a concentration of about 1×10^9 per ml, and appropriate dilutions of the two types of phage were plated, using the protrotrophs as "plating bacteria". Phage from λS plaques on recombinant bacteria was replated on C and K12S to determine the modifying ability of the recombinants.

Morphological features of the recombinant strains were observed on living cells with the phase contrast microscope. The nuclear morphology was studied with the electron microscope by the method described by Kellenberger

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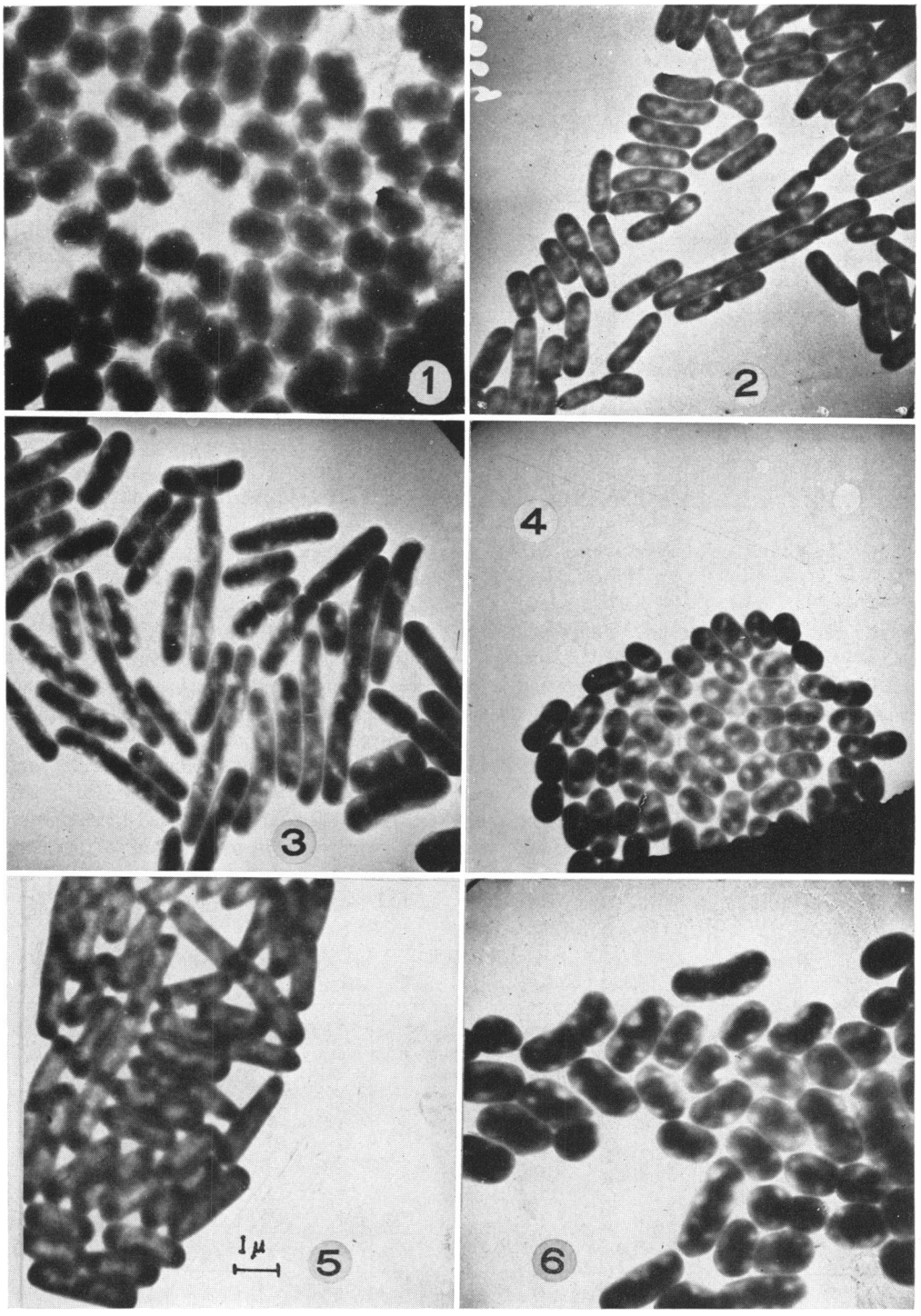

Figures 1-6. Electron micrographs of cells of various strains.

Figure 1. C parent. *Figure 2.* K12S parent. *Figure 3.* Recombinant with unselected markers of both parents. Shape like K12S and nuclear morphology like C. *Figure 4.* Recombinant with unselected markers of both parents. Shape intermediate between C and K12S and nuclear morphology like K12S. *Figure 5.* Recombinant with unselected markers from C only, but with cell shape and nuclear morphology like K12S. *Figure 6.* Recombinant with unselected markers from K12S only, but with cell shape intermediate between those of S and C and nuclear morphology like C.

TABLE 1

Prototrophs from crosses C × K12S

Parents in Cross	Total Number of Prototrophs Tested	Prototrophs with Unselected Markers from		
		C only	K12S only	C and K12S
(C)Pr$^-$ IV$^+$ V$_1^s$ V$_6^s$ (K12S)Pr$^+$ IV$^-$ V$_1^r$ V$_6^r$	74	68	5	1
(C)Pr$^-$ H$^+$ V$_1^s$ S^s (K12S)Pr$^+$ H$^-$ V$_1^r$ S^r	94	84	4	6
(C)Pr$^-$ H$^+$ V$_1^s$ Lac$^+$ (K12S)Pr$^+$ H$^-$ V$_1^r$ Lac$^-$	59	55	1	3
Total.....	227	207	10	10

(1952). The living cells were deposited by a filtration process directly on the collodion support. After fixation in vapors of osmium tetroxide, the cells were exposed to water, which reveals the nuclear bodies. The morphological features revealed by this method have been shown to correspond exactly to those obtained with the usual staining procedure for nuclear bodies.

RESULTS AND DISCUSSION

As shown in table 1, the majority of prototrophs recovered from the crosses carried unselected markers from the C parent only. The C parent is the F$^-$ or accepting partner in the cross (Lederberg, Cavalli, and Lederberg, 1952; Bertani, *private communication*). All the prototrophs with characters from both parents and a random sample of the other two types were examined for the presence of the five characters described in the introduction. Table 2 gives our findings.

The λ-accepting abilities (and S13 sensitivity) of the recombinants were indistinguishable from those of the C parent in quantitative tests. The λ-modifying ability also appeared to be the same in the recombinants and the parental C. However, many of the recombinants were intermediate between C and K12S in morphology. Strains were found with the rod shaped form of K12S but with nuclear bodies like C in shape and arrangement, and also strains, with round cells like C but with the rod shaped central nuclear bodies of K12S (figures 3 and 4). Prototrophs with markers from only one parent may inherit the morphology of the other parent (figures 5 and 6).

The fact that the λ-modifying and accepting abilities were always linked suggests that these properties may be inherited as a unit. This notion is strengthened by the absence of recombinants with accepting or modifying abilities intermediate between those of the C and the K12S parents, which would be expected to be present if several recombinable factors controlled these properties. The independent assortment of factors controlling cell form and the arrangement of the nuclear bodies suggests that these characters are not

TABLE 2

Properties of prototrophs from crosses C × K12S

Markers from	Cell Shape like	Nuclei like	Phage Modifying Ability like	Phage Acceptance Ability like	S13 Sensitivity like	
K12S and C	K12S	C	C	C	C	Figure 3
K12S and C	Intermediate	C	C	C	C	
K12S and C	Intermediate	C	C	C	C	
K12S and C	Intermediate	K12S	C	C	C	Figure 4
K12S only	Intermediate	C	C	C	C	Figure 6
K12S only	Intermediate	K12S	C	C	C	
C only	C	C	C	C	C	
C only	C	C	C	C	C	
C only	K12S	K12S	C	C	C	Figure 5
K12S only	K12S	K12S	K12S	K12S	K12S	Probably reversions
K12S only	K12S	K12S	K12S	K12S	K12S	

closely linked. In addition, they are apparently not closely linked to the λ-modifying and accepting abilities.

SUMMARY

The majority of the prototrophic recombinants in a cross between *Escherichia coli* strain K12S (F^+) and strain C (F^-) carry the unselected phage resistance, streptomycin and lactose fermentation markers of the C parent. Many of the recombinants were morphological hybrids of the two parental strains; cell shape and nuclear morphology behaved like independent genetic characters.

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